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Award Number: DAMD17-97-1-7279

TITLE: Correlative Study of Tumor Hypoxia and Metastatic Potential in Breast Cancer

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CONTRACTING ORGANIZATION: University of North Carolina

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13. ABSTRACT *(Maximum 200 Words)*

Purpose: To study if tumor hypoxia is associated with metastatic potential in breast carcinoma.

Scope: Breast cancer patients enrolled in an IRB approved study receive hypoxia marker pimonidazole intravenously. Tumor biopsy specimens are examined for pimonidazole binding (hypoxia) and for the bimolecular markers. Regional node metastases data are recorded.

Major findings: To date, 19 patients have been enrolled on the study. Tumor hypoxia detected by pimonidazole ranges from 0-33% by an image analysis system. A semi-quantitative grading scale of 0-4 (0=no hypoxia, 4=highest amount of hypoxia) developed in cervix cancer studies and validated with the image analysis method is also useful in breast cancer hypoxia assessments. There have been no pimonidazole toxicities. Preliminary data of tumor hypoxia and microvessel density have been (Appendix II-V).

Status and Progress Report (in terms of results and significance): This is the first demonstration of tumor hypoxia detection in human breast cancer using pimonidazole. These data suggest a valuable role for correlative studies of tumor hypoxia with both clinical and bimolecular markers of tumor aggressiveness. . It is early to perform correlative studies of tumor hypoxia, axillary node metastases and other bimolecular markers, pending further accrual of patients on the study.

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Annual Report Statement

Grant DAMD17-97-1-7279

INTRODUCTION

Background: Tumor hypoxia predicts for poor prognoses in human tumors independently of whether chemotherapy, radiotherapy or surgery is used as treatments. The hypoxic tumor microenvironment may be a significant factor in cellular processes involved in tumor proliferation and metastases. It has been postulated that this may be due to the induction of oxygen regulated proteins such as vascular endothelial growth factor (VEGF).

Purpose: A novel physiological approach using pimonidazole for tumor hypoxia assessment in human breast tumors has been initiated in this clinical study. The purpose is to study its association with metastatic potential of breast cancer cells. Does the presence of hypoxia in the primary breast tumors detected by pimonidazole immunohistochemical binding correlate with the presence of axillary lymph node metastases? Is tumor hypoxia associated with the presence of markers of cell proliferation (PCNA), microvessel density/VEGF, p53, and apoptosis in the primary breast tumor tissue.

Scope: The specific aims are:

I: Determine the presence and extent of tumor hypoxia in biopsies of primary breast cancer using pimonidazole binding to hypoxic tumor cells.

II: Determine the patterns of pimonidazole binding in the breast cancer biopsies in relation to other landmarks such as blood vessels and necrosis.

III: Correlate the presence and extent of tumor hypoxia in primary breast cancer with the presence of axillary node metastases.

IV: Correlate the presence and extent of tumor hypoxia with the presence of other biological markers such as micro-vessel density/VEGF, p53, apoptosis, and PCNA.

V: Monitor the adverse effects of pimonidazole.

Methods: Breast cancer patients enrolled in an IRB approved study receive pimonidazole intravenous infusion. Breast tumor biopsy specimens are examined with immunohistochemical techniques for pimonidazole binding (hypoxia) and for the above bimolecular markers. Regional node metastases data are recorded.

Research Progress Associated with Tasks outlined in the Statement of Work.

Task 1: Patient enrolment

To date, 19 breast cancer patients have been enrolled on Institutional Review Board (IRB) approved protocol for this DOD clinical study. Age and ethnic origin data are shown below.

Ages	Number
18-30	0
31-40	2
41-50	5
51-60	6
61-70	3
71-80	2
81-90	1

Ethnic Origin	
White	13
African-American	6

For each patient enrolled:

Task 1A	
a. Document eligibility criteria upon enrollment.	Done.
b. Have signed informed consent on record prior to study procedures.	Done
c. Record clinical data.	Done
Task 1B	
a. Administer pimonidazole, 0.5 g/m^2 in 100 ml N-saline i.v. *2 patients did not return for the pimonidazole infusions.	Done*
b. Procure breast biopsy specimen at the time of lumpectomy/mastectomy.	Done
c. Prepare biopsy material for pimonidazole and biological markers staining.	Done
d. Record pathological status of axillary lymph nodes.	On-going
Task 1C	
a. Perform pimonidazole immunostaining.	On-going
b. Perform p53, apoptosis, PCNA, Ki-67, and VEGF marker staining	Awaiting batch study
c. Record any adverse effects of pimonidazole	Done
Task 1D	
a. Perform Image Analysis on stained slides.	In progress
b. Calculate Hypoxic Fraction (ratio of labeled to unlabeled cells).	In progress
c. Record p53, apoptosis, PCNA, Ki-67, and VEGF marker staining.	Awaiting batch study
b. Assess hypoxia marker binding relationships to anatomic landmarks.	Awaiting batch study
Task 2 Perform Data and Statistical Analysis to obtain Results of Specific Aims I-V	See preliminary data below

Preliminary Data and Results

Pimonidazole labeling of breast cancer cells has been demonstrated in the tumor biopsy specimens of the patients enrolled in this DOD supported research study. Figure 1 (Appendix I) shows the labeling of hypoxic cells by pimonidazole. It is important to note that the adjacent necrotic (anoxic) cells are not labeled. Preliminary results have been combined with the results from 16 breast cancer patients enrolled in our own IRB approved study and presented at various meetings in the past year. Copies of the abstract are attached in Appendix II-V.

As reported previously there were administrative problems with regard to the approval of the consent form by the IRBs involved. After the initial award (IRB) of the grant, a number of changes in the Consent Form approved by our Institutional Review Board were requested by the Surgeon General's Human Subjects Research Review Board (HSRRB, HURRAD Log. No. A-7766), USAMRMC Human Subjects Protection Division. Following further discussions on this subject, language acceptable to both Review Boards was developed whereby Department of Defense as the sponsor of the research assumes the financial responsibility. These deliberations and required approval of the Consent Form has delayed entry of research subjects entry into the research protocol for this grant. There was a significant time lapse in obtaining agreement and approvals of the Informed Consent Form from the Institutional Review Boards for participation by breast cancer patients in the research study funded by this grant.

As a result patient enrolment was delayed and is gradually progressing. To address the patient recruitment requirements, a Research Nurse is involved in patient recruitment and assists with the clinical procedures involved in pimonidazole infusions, patient follow-ups, and clinical data management.

KEY RESEARCH ACCOMPLISHMENTS AND REPORTABLE OUTCOMES:

The study is ongoing and results are updated as more breast cancer patients are enrolled on the study. The following significant observations from the preliminary results:

1. Pimonidazole detects hypoxic regions in human breast carcinoma (Appendix I, Figure 1).
2. Innovative correlative study of tumor hypoxia detection with microvessel density analysis has been performed using a double staining technique on the tumor section on the same slide.
3. Abstracts are provided in Appendix II, III and III.
4. Dr. Ballenger was awarded a Travel Fellowship by the Radiation Research Society to present the results from this research at their April 2000 meeting.
5. Dr. Ballenger's research work on this project has been accepted as the research requirement for her Radiation Oncology training in the University of North Carolina Hospitals Residency Program.
6. Dr. Ballenger has been recruited to a faculty position in the Department of Radiation Oncology at Duke University Medical Center, Durham, North Carolina.
7. Consent form has been revised this year to meet the new format requirements of the IRB at UNC School of Medicine (Appendix VI).

CONCLUSIONS:

This is the first report of tumor hypoxia detection using pimonidazole immunohistochemical method in human breast cancers (Appendix II). Dr. Ballenger has been provided with research training and her contributions have led to 3 abstracts and a Travel Award Fellowship of the Radiation Research Society. Results from this breast cancer research will be updated as more patients are entered on the study and further analysis of the tumor biopsies is completed. There have been no adverse effects related to the pimonidazole infusions in these patients.

REFERENCES: Not Applicable.

APPENDICES

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples of appendices include journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Appendix I. Figure 1. Breast tumor biopsy from a patient shows hypoxic tumor cells immunostained with pimonidazole (Pimo) adjacent to micorvessels (V). Note that the necrotic cells do not show pimonidazole immunostaining.

Appendix II Ballenger CA, Varia MA, Chou S-C, Novotny DB, Haroon Z and Raleigh JA Hypoxia and microvessel Density in human breast adenocarcinomas. 22nd Annual San Antonio Breast Cancer Symposium, San Antonio, Texas, Dec 8-11, 1999.

Appendix III. Ballenger CA, Chou S-C, Calkins-Adams DP, Novotny, DB, Raleigh JA, Varia MA. Hypoxia And Microvessel Density In Human Breast Adenocarcinomas. American Radium Society Meeting, April 1-5, 2000, London, United Kingdom.

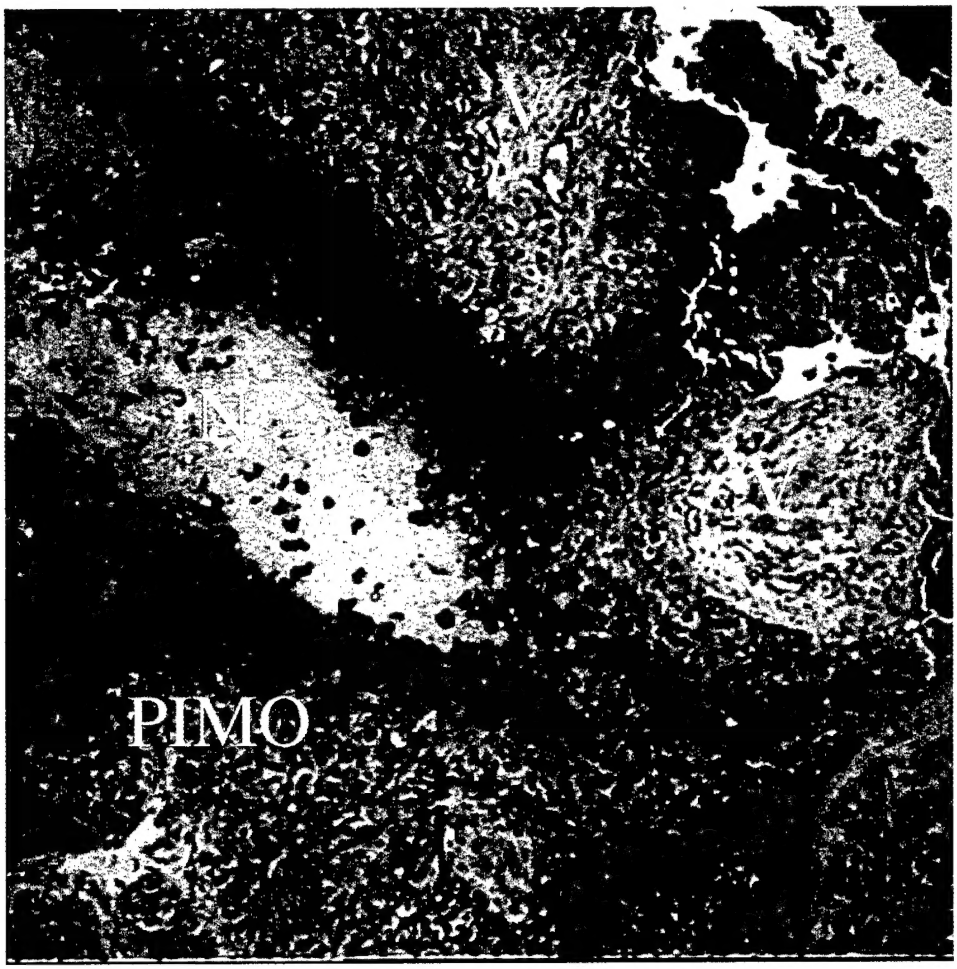
Appendix IV. Hypoxia and microvessel density in human breast adenocarcinomas. [1]Ballenger CA, [1]Varia MA, [1]Chou S-C, [2]Novotny DB, [3]Haroon Z and [1]Raleigh JA.. Radiation Research Society Meeting, Albaquerque, New Mexico, April 29-May 3, 2000.

Appendix V. Demonstration of Hypoxic Regions with Pimonidazole in Human Breast Cancers and Relationship to Microvascular Density. [1]Varia MA, [1]Ballenger CA, , [1]Chou S-C, [2]Novotny DB, [3]Haroon Z and [1]Raleigh JA. [1]Radiation Oncology and [2]Pathology, UNC School of Medicine, Chapel Hill, NC 27599; [3]Radiation Oncology, Duke University Medical Center, Durham, NC 27710.. Era of Hope meeting, Atlanta, June 8-11, 2000.

Appendix VI Revised Patient Consent Form.

Appendix I.

Figure 1. Breast tumor biopsy from a patient shows hypoxic tumor cells immunostained with pimonidazole (Pimo) adjacent to micorvessels (V). Note that the necrotic cells (N) do not show pimonidazole immunostaining.



APPENDIX II

Hypoxia And Microvessel Density In Human Breast Adenocarcinomas.

[1]Ballenger CA, [1]Varia MA, [1]Chou S-C, [2]Novotny DB, [3]Haroon Z and [1]Raleigh JA.

[1]Radiation Oncology and [2]Pathology, UNC School of Medicine, Chapel Hill, NC 27599; [3]Radiation Oncology, Duke University Medical Center, Durham, NC 27710.

.22nd Annual San Antonio Breast Cancer Symposium, San Antonio, Texas, Dec 8-11, 1999.

Tumor hypoxia predicts for poor prognoses in human tumors independently of whether chemotherapy, radiotherapy or surgery is used as treatments. It has been postulated that this is due to the induction of oxygen regulated proteins such as vascular endothelial growth factor (VEGF). However, VEGF is not expressed in the majority of hypoxic cells in human tumors so an examination of a product of VEGF action, microvascularization, has been examined as a possible link between hypoxia and poor prognosis. Microvascular density (MVD) has been measured in 16 patients with breast adenocarcinomas. Immunostaining for pimonidazole adducts was used to detect hypoxia and immunostaining for Factor VIII or transglutaminase were used to detect microvasculature. In the case of MVD, 5 fields (0.196 um^2) of highest MVD in a section from each of 4 biopsies per tumor were selected and scored. Isolated clusters of endothelial cells, single endothelial cells and branching structures were counted as one vessel. Factor VIII and transglutaminase gave similar results and interobserver agreement was excellent. Considerable intrabiopsy and interbiopsy variation was observed. MVD ranged from 2 to 82 vessels per field. Overall, high MVD correlated with low hypoxia ($p = 0.001$). We conclude that hypoxia does not control microregional vascular density but, instead, tumor vascularity determines the extent of hypoxia in human breast adenocarcinomas. We further conclude that the generation of high, microregional MVD is not the mechanism by which hypoxia leads to poor prognoses measured at the time of clinical presentation. This does not rule out the possibility that hypoxia plays a role in angiogenesis at earlier stages in the natural history of breast tumors. Supported by DHHS R 42 CA68826 and DOD BC962506.

Appendix III

Hypoxia and microvessel density in human breast tumors.

[1]Ballenger CA, [1]Chou S-C, [1]Calkins-Adams DP, [2]Novotny, DB, [1]Raleigh JA, [1]Varia MA.

[1]Radiation Oncology and [2]Pathology, UNC School of Medicine, Chapel Hill, NC 27599.

American Radium Society Meeting, April 1-5, 2000, London, United Kingdom.

ABSTRACT:

It has been reported that tumor hypoxia predicts for poor prognoses in human tumors independently of whether chemotherapy, radiotherapy or surgery are used to treat the tumors. Given the possibility that tumor hypoxia leads to poor prognosis by stimulating microvasculature, microregional hypoxia and microvascular density (MVD) has been measured in 16 patients with breast adenocarcinomas.

Immunostaining for pimonidazole adducts was used to measure hypoxia and immunostaining for Factor VIII was used to measure microvasculature. In the case of MVD, 5 fields (0.196 mm^2) of highest MVD in sections from 4 biopsies per tumor were selected and scored. Isolated clusters of endothelial cells, single endothelial cells and branching structures were counted as one vessel.

MVD ranged from 2 to 82 vessels per field. Considerable intrabiopsy and interbiopsy variation was observed. Overall, high MVD correlated with low hypoxia ($p = 0.001$). That is, hypoxia does not control microregional vascular density. Rather, tumor vascularity determines the extent of hypoxia in human breast adenocarcinomas.

We conclude that the generation of high, microregional MVD is not the mechanism by which hypoxia leads to poor prognoses measured at the time of clinical presentation. This does not rule out the possibility that hypoxia plays a role in angiogenesis at earlier stages in the natural history of tumors.

Supported by DHHS R 42 CA68826 and DOD BC962506.

Appendix IV

Hypoxia And Microvessel Density In Human Breast Adenocarcinomas.

[1]Ballenger CA, [1]Varia MA, [1]Chou S-C, [2]Novotny DB, [3]Haroon Z and [1]Raleigh JA.

[1]Radiation Oncology and [2]Pathology, UNC School of Medicine, Chapel Hill, NC 27599; [3]Radiation Oncology, Duke University Medical Center, Durham, NC 27710.

Radiation Research Society Meeting, Albuquerque, New Mexico, April 29-May 3, 2000

ABSTRACT

Tumor hypoxia predicts for poor prognoses in human tumors independently of whether chemotherapy, radiotherapy or surgery is used as treatments. It has been postulated that this is due to the induction of oxygen regulated proteins such as vascular endothelial growth factor (VEGF). However, VEGF is not expressed in the majority of hypoxic cells in human tumors so an examination of a product of VEGF action, microvascularization, has been examined as a possible link between hypoxia and poor prognosis. Microvascular density (MVD) has been measured in 16 patients with breast adenocarcinomas. Immunostaining for pimonidazole adducts was used to detect hypoxia and immunostaining for Factor VIII or transglutaminase were used to detect microvasculature. In the case of MVD, 5 fields (0.196 um^2) of highest MVD in a section from each of 4 biopsies per tumor were selected and scored. Isolated clusters of endothelial cells, single endothelial cells and branching structures were counted as one vessel. Factor VIII and transglutaminase gave similar results and interobserver agreement was excellent. Considerable intrabiopsy and interbiopsy variation was observed. MVD ranged from 2 to 82 vessels per field. Overall, high MVD correlated with low hypoxia ($p = 0.001$). We conclude that hypoxia does not control microregional vascular density but, instead, tumor vascularity determines the extent of hypoxia in human breast adenocarcinomas. We further conclude that the generation of high, microregional MVD is not the mechanism by which hypoxia leads to poor prognoses measured at the time of clinical presentation. This does not rule out the possibility that hypoxia plays a role in angiogenesis at earlier stages in the natural history of breast tumors. Supported by DHHS R 42 CA68826 and DOD BC962506.

Appendix V.

Demonstration of Hypoxic Regions with Pimonidazole in Human Breast Cancers and Relationship to Microvascular Density.

[1]Varia MA, [1]Ballenger CA, , [1]Chou S-C, [2]Novotny DB, [3]Haroon Z and [1]Raleigh JA. [1]Radiation Oncology and [2]Pathology, UNC School of Medicine, Chapel Hill, NC 27599; [3]Radiation Oncology, Duke University Medical Center, Durham, NC 27710.

BACKGROUND: Tumor hypoxia predicts for poor prognoses in human tumors independently of whether chemotherapy, radiotherapy or surgery is used as treatments. It has been postulated that this is due to the induction of oxygen regulated proteins such as vascular endothelial growth factor (VEGF).
OBJECTIVES: The purpose of this study is to demonstrate hypoxia in human breast cancer and study its relationships to biomarkers and clinical predictors of poor prognosis. However, VEGF is not expressed in the majority of hypoxic cells in human tumors so an examination of a product of VEGF action, microvascularization, has been examined as a possible link between hypoxia and poor prognosis.
METHODS: Detection of tumor hypoxia has been correlated with microvascular density (MVD) in 16 patients with breast adenocarcinomas entered on IRB approved protocols. Immunostaining for pimonidazole adducts was used to detect hypoxia and immunostaining for Factor VIII or transglutaminase were used to detect microvasculature. In the case of MVD, 5 fields (0.196 um^2) of highest MVD in a section from each of 4 biopsies per tumor were selected and scored. Isolated clusters of endothelial cells, single endothelial cells and branching structures were counted as one vessel. Factor VIII and transglutaminase gave similar results and interobserver agreement was excellent. MVD ranged from 2 to 82 vessels per field.
RESULTS: Pimonidazole detected the presence of hypoxic cells and the extent of hypoxia was quantified with image analysis method. Overall, high MVD correlated with low hypoxia ($p = 0.001$). Furhter analyis are in progress pending acrual of additional patients on the study.
CONCLUSIONS: These preliminary data demonstrates the presence of hypoxia in human breast cancers and that hypoxia does not control microregional vascular density but, instead, tumor vascularity determines the extent of hypoxia in human breast adenocarcinomas. This does not rule out the possibility that hypoxia plays a role in angiogenesis at earlier stages in the natural history of breast tumors.

Supported by DHHS R 42 CA68826 and DOD BC962506.

between 6-30-00 and 6-30-01 signed only

APPENDIX VI

of Medicine IRB

GCRC Protocol: 1425-GRC

Revised Patient Consent Form

UNC
Chapel Hill, North Carolina
UNC-CH Study Number: GCRC

Name and Hospital Number:

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

1. Title of Research Study: Correlative Study Of Tumor Hypoxia And Metastatic Potential In Breast Cancer

2. Sponsor Study Number: U. S. Department of Defense Grant No. DAMD 17-97-1-7279

3. Principal Investigator: Mahesh A. Varia, M. D. PHONE: 919-966-7700

4. Co-Investigators: James Raleigh, Ph.D., Cynthia Ballenger, M.D., Debra Novotny, M.D., William Cance, M.D., Mark Graham, M.D.

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5. You are asked to participate in a research study under the direction and medical supervision of Mahesh A. Varia, M.D. Other professional persons who work with him may assist him or act on his behalf. The purpose of this research is to detect tumors with low oxygen using a drug called pimonidazole and to study the growth aspects of tumors that contain cells with low oxygen.

   The Food and Drug Administration (FDA) has approved the use of pimonidazole as an Investigational New Drug (IND No. 036,783). Investigational New Drug approval does not mean that the drug is approved for routine use.

   You have the option not to participate in this research study if you so desire. You have been asked to participate in this study because you have been diagnosed with cancer. We would like to study the presence of low amounts of oxygen in cancer and its relationship with other growth and spread features of the cancer.

   You will be one of approximately 50 patients in this research study being conducted at UNC Hospitals. Similar studies using pimonidazole for other types of cancers are being conducted at the UNC Hospitals and at other medical centers in USA and Europe .

6. PURPOSE : The purpose of this research study is:

   a. To identify cancers cells with low oxygen (hypoxic cells)

   b. To study the relationships between such hypoxic cells and the growth and spread features of cancer cells and

   c To monitor the side effects of pimonidazole.

   You understand that no treatment benefit is expected by participating in this study.

   It is known that hypoxic cells (cells with low amounts of oxygen) can survive the damaging effects of radiation. This could be one reason why some cancers are not cured by radiation therapy. We would like to study whether hypoxic cells are associated with a greater risk of the spread of cancer cells. Information from this type of study will improve our understanding of cancer and cancer treatment.

7. DURATION: Your participation in this study will last for approximately 4 months that includes the follow-up examinations. Your medical care will be continued with your doctors.

8. PROCEDURES: You are asked to participate in a procedure that involves giving you pimonidazole through the vein (I.v. or intravenously). This drug is prepared as a liquid solution. It has the special property of attaching to cells with low amounts of oxygen. By obtaining a sample of the tumor (biopsy) and doing special tests on this sample in the laboratory, we wish to find out if the tumor contains cells with low amounts of oxygen. Other tests on the tumor sample will be done to find out the growth behavior of the cancer cells. In addition, you will receive physical examinations, blood, urine and laboratory tests, and biopsy of the suspected cancer tissue as a part of this study.

The biopsy procedure for this study may be performed in the clinic or at the time of surgery for the management of your cancer, as advised by your treating physicians.

You understand that there are possibilities that the blood, urine, biopsy and tissue samples which you are providing under this study may also be used in other research studies and could potentially have some commercial applicability.

You understand that a serum pregnancy test will be performed on all female subjects of childbearing potential within 48 hours prior to the pimonidazole infusion. You understand that you should avoid becoming pregnant for at least one week after the pimonidazole infusion. To avoid becoming pregnant, you should abstain from sexual relations or practice a method of birth control. Except for surgical removal of the uterus, birth control methods such as the use of condoms, a diaphragm or cervical cap, birth control pills, IUD, or sperm killing products are not totally effective in preventing pregnancy.

During the course of this study the following will occur:

You will have tests to check your blood counts, liver and kidney functions, and urine examination. These are similar to the blood and urine tests done when patients are admitted to the hospital.

You will be given one dose of pimonidazole (0.5 to 1 gram depending upon your weight and height) through a vein over 20 -30 minutes. This will take place on the day before your planned surgery. This will be done either as an inpatient in the General Clinical Research Center or in the outpatient clinics of the UNC Hospitals according to your wishes.

You will have a biopsy procedure to obtain samples of the known or suspected cancer tissue. The size of the sample will be determined in consultation with your surgeon and the pathologist-in-charge or the clinical laboratory director. Usually, this sample will be small. Excess tissue from the surgical specimen may be made available for this study.

These tissues will be tested in the laboratory with special tests designed to detect hypoxic cells and some of the other growth features of the tumor.

You will be asked to return for a medical checkup to monitor any side effects in approximately 2 - 4 months after the Pimonidazole injection. You will also have blood and urine tests to check your blood counts as well as your liver and kidney functions .

Approximately 20 cc (about 4 teaspoonfuls) of blood will be drawn during the initial part of the study, and about 9 cc (about 2 teaspoonfuls) of blood will be drawn at each of the follow-up visits.

You will be asked to report any side effects of the Pimonidazole, the biopsy procedures, or any other side effects experienced during the study period.

9. EXCLUSIONS: You should not participate in this study if any one of the following applies to you:

a. You had severe infection in the past 4 weeks.
b. You have nerve damage from chemotherapy or other causes.
c. You believe you are pregnant.
d. You are completely disabled and cannot perform personal self care.
e. You have received another investigational or experimental drug in the past 4 weeks.

10. DISCOMFORTS AND RISKS: Although this study is designed to cause few, if any, side effects, the following risks and /or discomforts are possible:

While pimonidazole is an investigational new drug in the USA, it has already been studied for its side effects in the United Kingdom. Pimonidazole doses higher than those used in the present study were found to be well-tolerated in patients in the United Kingdom. Very few, if any, side effects are expected from the amount of Pimonidazole that will be used for you in this study. No significant side effects have been observed in 50 patients who have received pimonidazole at the University of North Carolina.

Possible side effects include nausea, vomiting, a sensation of heat, and sweating. If these side effects do occur, they are usually reversible. Other side effects of Pimonidazole such as skin rashes and mental disorientation are unusual, but if they do occur, symptomatic treatment will be available.

Blood Tests and Biopsy Procedures: Risks associated with these procedures include bleeding, infection, surgical injury and discomfort. However, biopsies are common medical procedures with a very low risk of complications. Biopsies will be performed by qualified personnel and are not expected to increase risks or discomfort beyond those of your planned surgery itself. Special studies to be performed in the laboratory on the tissue samples obtained will not confer any additional risks to you.

11. UNFORSEEABLE RISKS: Some risks/discomforts are unforeseeable.

12. BENEFITS: No direct treatment benefits are anticipated for you by participating in this study because it does not involve treatment of your cancer. However your participation may lead to a better understanding of the role of hypoxic cells and other growth characteristics of tumors. This knowledge may assist in the treatment of future cancer patients.

13. ALTERNATIVES: This study does not provide treatment for your cancer and you have the option not to participate in this study.

14. NEW FINDINGS: You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

15. CONFIDENTIALITY: Every effort will be taken to protect the identity of the participants in this study. However, there is no guarantee that the information cannot be obtained by legal process or court order. No subjects will be identified in any report or publication of this study or its results.

Your records will be reviewed and analyzed by physicians, other personnel and their designees associated with this study. Your medical records may be audited by the representatives of the UNC School of Medicine, the UNC Hospitals, the Food and Drug Administration (FDA), and applicable research grant funding agencies [National Institutes of Health (NIH) and National Cancer Institute (NCI)or the U.S. Army Medical Research and Material Command(USAMRMC)].

16. FINANCIAL COSTS OF THIS RESEARCH: You will not be billed for the research costs of your stay in the General Clinical Research Center, the blood tests, the biopsy procedure, the research laboratory tests done for this study, or for the follow-up visits and blood tests. You will not be billed for the cost of the Pimonidazole. Research grants from the NIH, NCI, USAMRMC, and the University of North Carolina cover these costs.

You are responsible for any costs not associated with this study, such as all tests, x-rays, drugs, physicians' fees, devices and hospital or clinic fees. You are responsible for your own transportation, and incidental expenses.

17. COMPENSATION IN CASE OF INJURY: In the event of any injury resulting directly from the research procedures, financial compensation cannot be provided by either the study personnel or the University of North Carolina at Chapel Hill. All forms of medical diagnosis, treatment and research, whether routine or experimental, involve some risk of injury. In spite of all precautions, you might develop complications from participation in this study. If such complications arise, the researchers will assist you in obtaining appropriate medical treatment, but the University of North Carolina at Chapel Hill does not provide financial assistance for medical or other costs. You do not waive any liability rights for personal injury by signing this form.

The United States Department of Defense is funding this research project. Should you be injured as a direct result of participating in this research project, you will be provided medical care, at no cost to you, for that injury. The cost of such medical care will be provided by the United States Department of Defense. You will not receive any injury compensation, only medical care. You should also understand that this is not a waiver or release of your legal rights. You should discuss this issue thoroughly with the Principal Investigator or his designee before you enroll in this study.

18. PAYMENTS TO PARTICIPANTS: For your participation in this study, you will receive $ 200 from a grant funded by the Department of Defense. A cheque for this amount will be sent to you by the Grant Manager in the Department of Radiation Oncology at the University of North Carolina at Chapel Hill.

19. RIGHT TO REFUSE OR TO WITHDRAW FROM THE STUDY Your participation in this study is voluntary. You may refuse to participate, or may discontinue your participation at any time without penalty, without jeopardizing your continuing medical care at this institution, and without losing benefits you would otherwise be entitled to. Dr. Mahesh A. Varia has the right to stop your participation in the study at any time. This could be because you had an unexpected reaction, failed to follow instructions, or because the study has been stopped.

20. INSTITUTIONAL REVIEW BOARD APPROVAL: This project has been approved by the Committee on the Protection of the Rights of Human Subjects at the University of North Carolina at Chapel Hill. If you believe that there is any infringement of your rights, you may contact the Chairman of this Committee, Ernest N. Kraybill, M.D. at 919-966-1344.

21. SUBJECT PARTICIPANT'S RESPONSIBILITY: By consenting to participate in this study, you are responsible for carrying out the instructions and you must relate to your doctors, nurses or other study personnel any information that might be pertinent to the study, such as any side effects of a treatment or procedure.

22. U.S. ARMY MEDICAL RESEARCH AND MATERIAL COMMAND POLICY (USAMRMC): It is the policy of the USAMRMC that data sheets are to be completed on all volunteers participating in research for entry into the USAMRMC's Volunteer Registry data base. The information to be entered into this confidential data base includes your name, address, social security number, study name and dates. The intent of the data base is twofold: first, to readily answer questions concerning an individual's participation in research sponsored by the USAMRMC; and second, to ensure that the USAMRMC can exercise its obligation to ensure research volunteers are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRMC for a minimum of 75 years.

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3. OFFER TO ANSWER QUESTIONS: I have had the opportunity to ask, and have had answered, all my questions about this research. If I have other questions, or if a research-related injury occurs, I will call Mahesh A. Varia, M.D. at 919-966-7700 or the Radiation Oncology physician on call at UNC Hospitals, phone 919-966-4131.

24. MEDICAL RECORDS REVIEW: I understand that the drug Pimonidazole is an investigational new drug and requires that the results of its use in patients be reported to the Food and Drug Administration. I agree to have my records reviewed by their representatives.

I understand that there is a possibility that the biopsy material and body fluids that I am providing for this study may also be used in other research studies, and may have some commercial applicability.

25. SUBJECT'S AGREEMENT: I have read the information provided above. I voluntarily agree to participate in this study. After this document is signed, I understand that I will receive a copy of this consent form.

Signature of Research Subject: _____ Date: _____

Name (Print or Type): _____

Signature of Witness: _____ Date: _____

Name (Print or Type): _____

Signature of Person Obtaining the Consent: _____ Date: _____

Name (Print or Type): _____

This consent form should be signed only between 6-30-00 and 6-30-01

Approved by School of Medicine IRB

UNC
Chapel Hill, North Carolina
UNC-CH Study Number: GCRC-1425-01RC

Name and Hospital Number

Sample Donation Form

Title of Research Study

Correlative Study Of Tumor Hypoxia And Metastatic Potential In Breast Cancer

I voluntarily and freely donate any and all blood, tissues, and body fluid samples such as urine obtained as part of this research study to the University of North Carolina at Chapel Hill, and hereby relinquish all right, title, and interest to said items.

Signature of Research Subject: _____ Date: _____

Name (Print or Type): _____

Signature of Witness: _____ Date: _____

Name (Print or Type): _____

Signature of Person Obtaining the Consent: _____ Date: _____

Name (Print or Type): _____